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Evaluation of Optimal Storage Temperature, Time, and Transport Medium for Detection of Group B Streptococcus in StrepB Carrot Broth

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The performances of the ESwab and Amies transport media were evaluated for optimal survival of group B streptococcus (GBS) in StrepB carrot broth. ESwab was superior to Amies at all temperatures evaluated but was optimal at 21°C and 24°C, whereas recovery in Amies was significantly decreased at these temperatures.

Group B streptococcus (GBS) is the leading infectious cause of morbidity and mortality among newborns in the United States (8). The incidence of perinatal GBS disease has declined significantly since the broad implementation of universal GBS screening and intrapartum antibiotic prophylaxis (IAP). An 80% decrease in incidences, from 1.7/1,000 live births in 1993 (7) to 0.34/1,000 live births in 2003 to 2005 (5), has been reported. Despite the declining rates in disease incidence, coincident with increased preventative strategies, a recent study reported a case fatality rate of 7.9% in the United States (5). In addition, in a study by Van Dyke et al. (10), 61.4% of infants with GBS disease were born to women who were GBS screen negative; this number is mostly representative of GBS screening by culture, since only 0.5% of the cases evaluated were screened by other methods, such as PCR. Overall, optimal detection of GBS colonization is paramount for the selective prescription of prophylaxis at the time of delivery.

In 2010, the Centers for Disease Control and Prevention (CDC) revised consensus guidelines for the prevention of early-onset GBS disease (11). In addition to the recommended universal screening of all pregnant women at 35 to 37 weeks gestation for vaginorectal colonization by a broth enrichment method, the 2010 guidelines expanded the recommendation to include identification by chromogenic medium and directly from enriched broth. Chromogenic enrichment broths (i.e., StrepB carrot broth and Granada Biphasic broth) used to detect beta-hemolytic GBS were also included in the current recommendation (11). StrepB carrot broth (Hardy Diagnostics, Santa Clara, CA) produces red-orange pigments in the presence of beta-hemolytic GBS, obviating the need for subsequent subculture and reducing detection and identification to a single step. All negative results from StrepB carrot broths require further subcultures to screen for nonhemolytic strains of GBS. Due to its reported increase in sensitivity and specificity (2, 3), StrepB carrot broth was the primary detection medium included in this study.

Optimal confirmation of GBS colonization is facilitated by the appropriate collection and transport of the clinical specimen to the laboratory. Swab systems have become increasingly important due to the delay of specimen transport necessitated by recent strategies of cost-containment and consolidation of laboratory services. Specimens may be in transit for long durations, and the integrity of the specimen may be compromised. CDC guidelines recommend the transport of swabs in a nonnutritive medium, such as Amies transport medium (Biomedics, Madrid, Spain), for up to 4 days at room (20 to 25°C) or refrigeration temperature (11). However, limited data are available to support this recommendation. One study demonstrates that viability of GBS is indeed preserved for up to 4 days at 3°C and 24°C when an inoculum of 10 or more organisms was used (9). However, another study found that the storage of swabs at 4°C and 21°C compromised the ability of the culture to detect GBS colonization, causing some GBS-positive samples to be lost after 24 h (6). A relatively new type of swab system has been recently introduced in a growing number of laboratories. ESwab (Copan, Murrieta, CA) is a nylon-tipped swab prepared by a spray-on flocked fiber technique designed to optimize collection and to minimize the entrapment of the specimen. This is in contrast to the Amies transport medium used in this study which consists of a soft rayon swab tip in a gel column containing a modified version of Stuart's original formula to further promote proliferation and survival of the organism (1). One study compared the ESwab with Amies for the recovery of GBS and found recovery to be significantly higher in ESwab than in Amies at room temperature (RT) and 4°C (4). Therefore, we sought to compare the capabilities of ESwab and the Amies transport system in preserving GBS at 4°C, 21°C, and 24°C for up to 6 days, using StrepB carrot broth for enrichment and detection.

A total of 50 isolates of hemolytic GBS recovered from vaginorectal swab specimens were evaluated in this study. Isolates were initially subcultured onto Colombia sheep blood agar (BA) and identified using the Prolex streptococcal grouping latex kit (Prolab Diagnostics, Austin, Texas) for rapid antigen detection. An initial organism suspension of 0.5 McFarland standards ($\sim 1 \times 10^8$ CFU/ml) in sterile saline was prepared for each isolate and initially diluted 1:10, followed by serial dilutions of 1:100 to achieve an inoculum concentration of approximately 1×10^3

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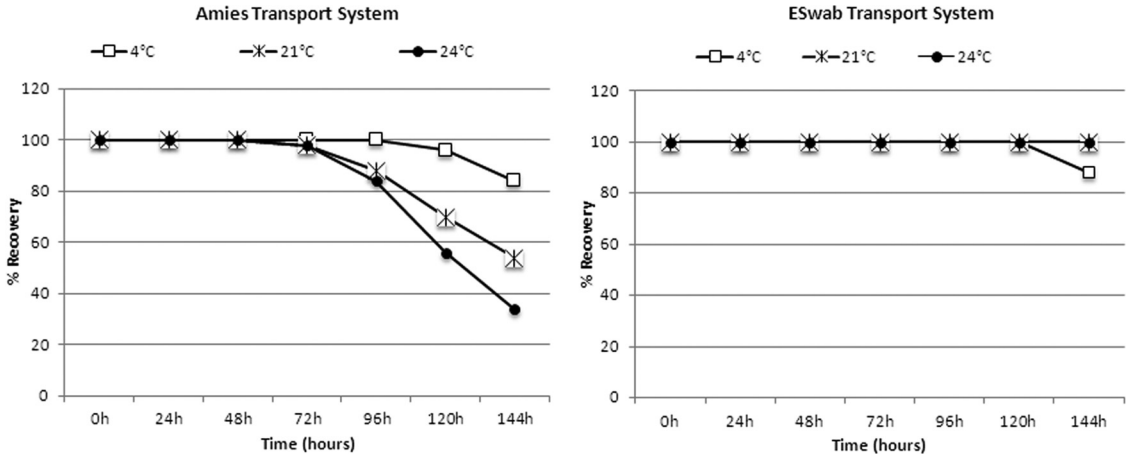

FIG 1 Recovery of group B streptococcus in two different transport media at different temperatures and incubation times. While recovery of group B streptococcus was optimal at 4°C using the Amies transport medium, the ESwab transport medium demonstrated optimal recovery at 21°C and 24°C, maintaining GBS viability for up to 6 days.

CFU/ml; prior dilution studies performed in our laboratory determined this dilution to be optimal for this study. One-hundred-microliter aliquots of the 10^3-CFU/ml suspension were inoculated onto one Amies swab for each time point ($n = 7$) and for each temperature ($n = 3$). The liquid medium in the ESwab made it possible to inoculate multiple culture plates with one system; thus, 100-μl aliquots of the 10^3-CFU/ml suspension were inoculated onto only one ESwab for each temperature ($n = 3$). All swabs were immediately placed into their respective transport systems and stored at controlled temperatures of 4°C, 21°C ± 1°C, or 24°C ± 1°C for each time period, up to 6 days (144 h). After each 24-h interval, the Amies swab and 100 μl of the ESwab transport medium were inoculated into StrepB carrot broths and incubated at 35°C and 5% CO^2 for 24 h. Zero-hour specimens were also included and were inoculated into StrepB carrot broths within 15 min of incubation at each temperature. Results were recorded as 0, 1+, 2+, 3+, and 4+, with the degree of pigmentation correlating with the positivity of the beta-hemolytic GBS recovered. Negative broths were further subcultured onto BA and incubated for 18 to 24 h in 5% CO^2 at 35°C, and colonies resembling GBS were confirmed by an antigen detection method. Any culture negative at 18 to 24 h was reincubated for a total of 48 h and screened for GBS again. A two-tailed Fisher exact test (GraphPad Prism 5; La Jolla, CA) was used to evaluate statistically significant differences in GBS survival when time, temperature, and transport systems were compared.

Storage time and temperature had a significant impact on the recovery of GBS from both transport media. When Amies transport medium was stored at 4°C, GBS recovery was 100% at 96 h and 84% at 144 h (Fig. 1). Recovery of GBS decreased significantly when Amies swabs were stored at 21°C and 24°C. At both temperatures, GBS recovery was 98% up to 72 h, followed by a significant decline in recovery, ranging from 34% to 54% at 144 h ($P = 0.0001$). Overall, GBS recovery using Amies transport medium was most sensitive at 4°C and was suboptimal at 21°C and 24°C, with 23/50 and 33/50 isolates having failed recovery at 144 h, respectively. Interestingly, performance of the ESwab transport medium far exceeded that of the Amies transport medium, particularly at RT conditions. When the ESwab transport medium was held at 4°C, GBS recovery was 100% up to 120 h and declined to 88% at 144 h (Fig. 1). There was no significant correlation between the isolates that failed to grow at 4°C in the Amies and ESwab transport media. Recovery of GBS remained stable at 100% when swabs were stored at 21°C and 24°C for the entire 144-h duration. Overall, GBS viabilities in the ESwab and Amies transport media were comparable when stored at 4°C, with 84% and 88% of GBS recovered, respectively. In comparison with the performance of the Amies transport medium at 144 h, GBS viability using the ESwab transport medium was significantly improved, with 100% of GBS isolates recovered at 144 h ($P = 0.0001$).

The difference in the degree of pigmentation of the positive StrepB carrot broth between the transport media was also determined (Fig. 2). The abilities to recover GBS at 4°C were similar for the ESwab and Amies transport media, with 42/50 and 44/50 isolates recovered at 144 h, respectively (Fig. 2a). Of the isolates that were positive for GBS at 144 h, 4 each were at high quantities (3+/4+) and 38 to 40 were at low quantities (1+/2+). At 21°C, GBS recovery was significantly greater when swabs were stored in ESwab than when they were stored in Amies (Fig. 2b). Storage in both media yielded full recovery of all 50 isolates up to 48 h, but the degrees of positivity were significantly different: 45/50 at high quantities for ESwab and 8/50 at high quantities for Amies. Furthermore, at 144 h and 21°C, 0 isolates were recovered at high quantities, 27 were recovered at low quantities, and 23 GBS isolates failed to grow when the Amies transport medium was used. In contrast, storage in the ESwab transport medium yielded 100% recovery at 144 h, with 43 recovered at high quantities and 7 recovered at low quantities. Storage at 24°C using the Amies and ESwab transport media yielded similar results, with all 50 isolates recovered up to 48 h, including 46 at high quantities for ESwab and 11 at high quantities for Amies (Fig. 2c). Amies transport medium at 144 h and 24°C resulted in 0 isolates at high quantities, 17 at low quantities, and 33 nonviable isolates. In contrast, all 50 GBS isolates were fully recovered in the ESwab transport medium, with 43 at high quantities and 7 at low quantities.

Optimal specimen collection and transport are essential for accurate laboratory diagnosis and timely treatment of GBS disease. Results from this study support the 2010 CDC guidelines that

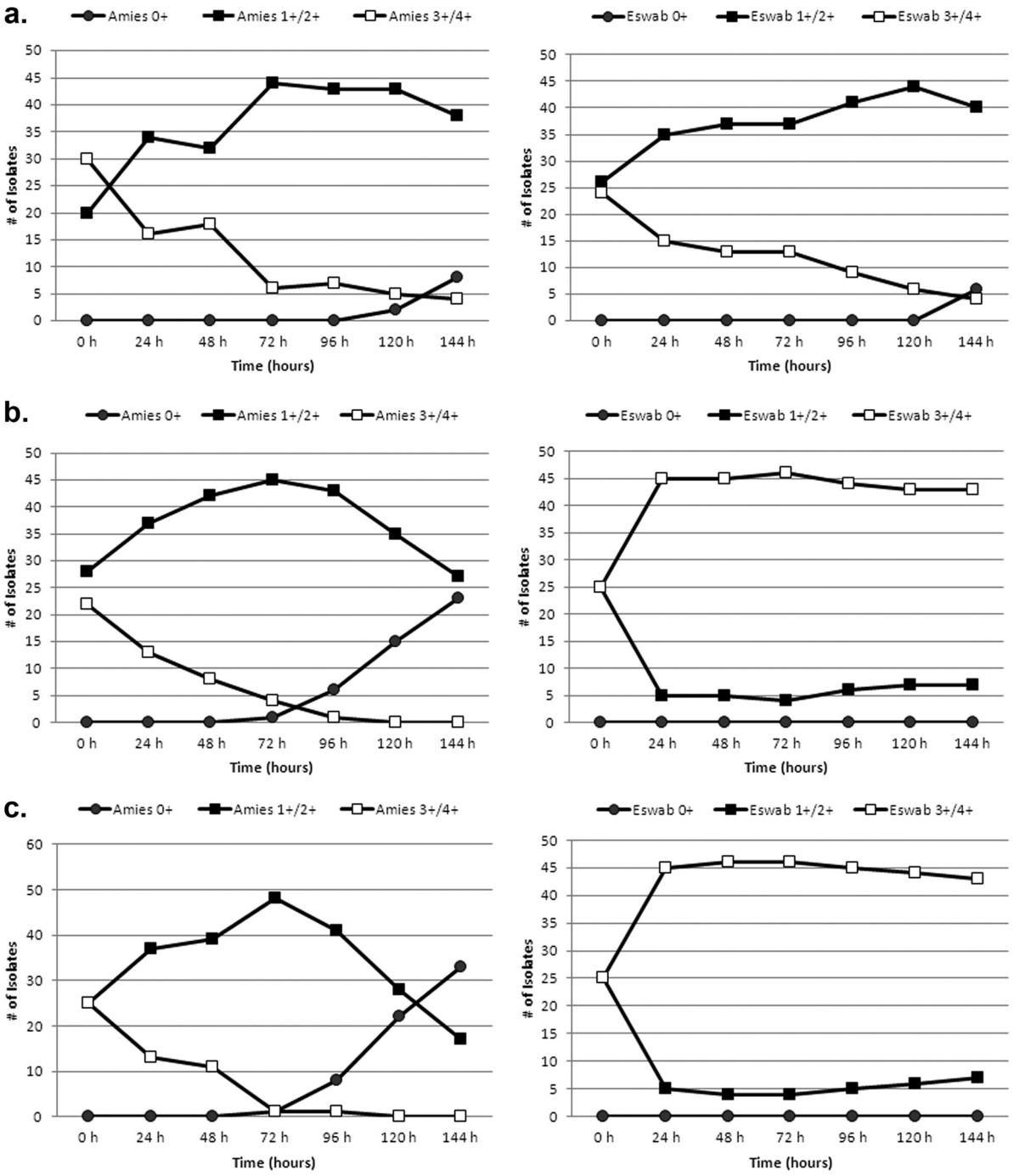

FIG 2 Degree of pigmentation of 50 group B streptococcus isolates incubated in StrepB carrot broth after storage in the Amies and ESwab transport systems for up to 144 h at 4°C (a), 21°C (b), and 24°C (c).

chromogenic enrichment broths, such as StrepB carrot broth, may be used as enrichment and detection broths for GBS. In addition, when a nonnutritive transport medium, such as Amies, is used, GBS isolates can remain viable for up to 4 days at room or refrigeration temperature. Interestingly, this study supports a previous study that demonstrated an impairment of GBS detection when the Amies transport system was stored at RT for extended periods of time (6). Therefore, vaginorectal swabs in Amies should be refrigerated, particularly in cases in which extended periods of transport cannot be avoided. Our results also confirm that ESwab is superior in the preservation of hemolytic GBS in StrepB carrot broth (4), particularly under RT conditions in which full GBS recovery was demonstrated after up to 6 days. In light of these findings, the CDC may consider expanding the recommendations to include alternate transport systems in addition to nonnutritive medium. However, further studies to compare the recoveries of nonhemolytic GBS isolates from the two transport systems are warranted.

Increased preventative strategies have considerably decreased perinatal GBS disease incidence, declining 80% from 1993 to 2005 (5, 7). The use of new methods with greater diagnostic efficiency may contribute even further to this improvement. Accordingly, this study compared the performances of Amies and ESwab transport media to detect GBS colonization in StrepB carrot broth and found ESwab to be far superior, especially at RT conditions.

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